Aquatic Toxicology

Editor
Lavern J. Weber

Volume 1

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Editor

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Preface

During the past two decades of environmental concern, there has been increasing interest in the impact of physical and chemical environmental changes to man and all other biological organisms. Studies on the effects of environmental perturbations have ranged from broad ecological investigations to studies on individual species. Our understanding of the influence of these changes on individual aquatic organisms has benefited from the existence of a broad knowledge base of physiology, biochemistry, pharmacology, and toxicology of terrestrial animals, especially mammals. By the early 1980s, the accumulated information on the responses of aquatic organisms to changes in their physical environment was extensive enough to justify a series of reviews of the state of this knowledge. *Aquatic Toxicology* provides these reviews.

This first volume covers the cardiovascular system and the many complexities that can be examined in the various fishes used in cardiovascular toxicology. The hepatic system is reviewed at the organ function level and then carried into a very specific mixed-function oxidase induction system in another chapter. One chapter is devoted to chemical carcinogenesis in fish because of the relative sensitivity of a fish model system for carcinogenesis.

Future volumes will include chapters on the respiratory system, renal system, nervous system, and specific toxicants, such as cyanide. They will also cover the areas of toxicological and comparative biomedical studies of aquatic invertebrates.

From an organizational standpoint, *Aquatic Toxicology* proceeds from the general to the specific. First to be examined is whole animal toxicology with emphasis on routes of administration and exposure to toxicants of concern in the environment. Next, the organ system level of organization is considered with reviews of specific func-

tional organ toxicity. Toxicological studies at the operational level are reviewed next. By operational, we refer to the effects that a toxicant has on very specific physiological and biochemical systems, such as enzymes, receptors, and membranes. Specific classes of toxicants will be reviewed in future volumes.

Although this text focuses primarily on aquatic animals, analogies to mammalian systems are included for comparison. Discrepancies of experimental conditions and the problems with the numerous species involved in the aquatic environment are indicated. The many problems of water quality conditions, including pH, temperature, hardness, alkalinity, and salt concentrations found in various areas are discussed.

The purpose of this book is to review the various organ systems and toxicological problems for investigators in the field and to cover the basics of the field for the biologist and biomedical researcher who intend to use aquatic organisms for model systems or very specific studies. It is intended to help educate the reader about the many peculiarities of the species involved. There are in excess of 20,000 species of fish, with life styles, habitats, morphology, and physiology more diverse within the fishes than in the most unusual species of mammals. An effort has been made to delineate many of these differences and the consequent problems in physiological, biochemical, pharmacological, and toxicological studies caused by this diversity.

These toxicological reviews cover whole animal, physiological, pharmacological, and biochemical levels of investigation of aquatic organisms. This volume will be of interest to all who utilize aquatic organisms in biomedical research of a toxicological nature.

Lavern J. Weber

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The Physiological Pharmacology and Toxicology of Fish Cardiovascular Systems

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This chapter is written for research students and other investigators who have a fundamental understanding of the pharmacology and physiology of mammalian cardiovascular systems and of fish cardiovascular anatomy, and who have an interest in initiating research on the pharmacology and toxicology of fish cardiovascular systems. It must be emphasized at the onset that there are more species of fish than there are species in all the other vertebrate classes combined; and fish have a great diversity of structure, reflecting the fact that the origins of different groups extend back from the Cretaceous to the Devonian. The broad definitions of pharmacology, as the study of the effects of chemicals on the function of these systems, and of toxicology, as investigations directed to those effects considered adverse to the organism, have been used (100).

After a brief introduction on fish cardiovascular structure and dynamics, with emphasis on the major pre- and postgill vasculature, attention is focused on a historical review of classic pharmacological analyses of cardiovascular responses to humoral compounds and neurotransmitters and, finally, on the use of fish cardiovascular responses in aquatic toxicology programs. Discussions of fish cardiovascular pharmacology have been limited to brief summaries in articles on fish physiology (114,140,158,159). A thorough review of this area of pharmacology is required, however, for at least two reasons. First, understanding the nature and magnitude of inherent

responses of physiological systems to chemicals is essential for providing accurate interpretation of results in toxicology. Second, methods and procedures developed in pharmacological analyses frequently guide the selection of approaches in toxicological investigations. Consequently, an appreciation of the types of responses and available approaches will assist the investigator who is initiating research in this area.

Comprehensive references are available for the reader interested in the broader aspects of fish cardiovascular physiology (140,158); in specialized areas, such as microcirculation (159); in processes involving the gill, such as gas exchange (78,141,193), acid-base balance (1,193), and osmoregulation (108); in the innervation of fish cardiovascular systems (24,157,159); and in comparative cardiorespiratory physiology of nonmammalian vertebrates (193).

CARDIOVASCULAR STRUCTURE AND DYNAMICS

With the exception of certain cyclostomes and air-breathing fish, there is a general conformity of structure in fish cardiovascular systems. The heart is divided into four chambers located in series in the order of the sinus venosus, atrium, ventricle, and conus arteriosus or, in teleost fish, the bulbus arteriosus. The electrocardiogram may exhibit as many as four deflections, one preceding the contraction of each of the four chambers (G. H. Satchell, *personal communication*). In most fish, the pacemaker site is in the sinus venosus (156), usually at the sinoatrial valve (71). The heart pumps venous blood and is located in a pericardium, which is generally rigid in elasmobranchs and flexible in teleosts (158). Flow is kept unidirectional by valves located at the sinoatrial and atrioventricular openings and at the junction of the ventricle and the conus or the bulbus. From the heart, blood is passed in sequence to the ventral aorta, with some alternative pathways through the branchial (gill) circulation for oxygenation, and then into the dorsal aorta. Alternative pathways to blood flowing through the branchial vasculature include a hypobranchial circulation including the pseudobranch, a

filamental circulation from the efferent filament arteries, and a nutritive circulation to the gill arch and interbranchial septum (170). From the dorsal aorta, which is the major postgill arterial vessel, blood goes to either the hepatic, the systemic and intestinal, or the renal capillary system. Venous blood finally collects in the sinus venosus and then returns to the heart. The heart pumps only venous blood and the coronary arteries arise, not from the aorta close to the valves, as in mammals, but from the efferent gill arteries that carry oxygenated blood. For a schematic representation of a salmonid heart and associated vasculature, see Fig. 1.

There are two basic variations to this general scheme. Hagfish have accessory hearts in their circulatory systems, as well as a larger blood volume and lower blood pressure (140). Lungfish have a pulmonary circulation and a partial separation of oxygenated and deoxygenated blood in their hearts (80).

The rest of this section will emphasize the physiology and structure of large blood vessels, since this has been a neglected area in recent reviews. A recent chapter by Satchell (159) provides a thorough review of capillary types and gill microcirculation. Detailed

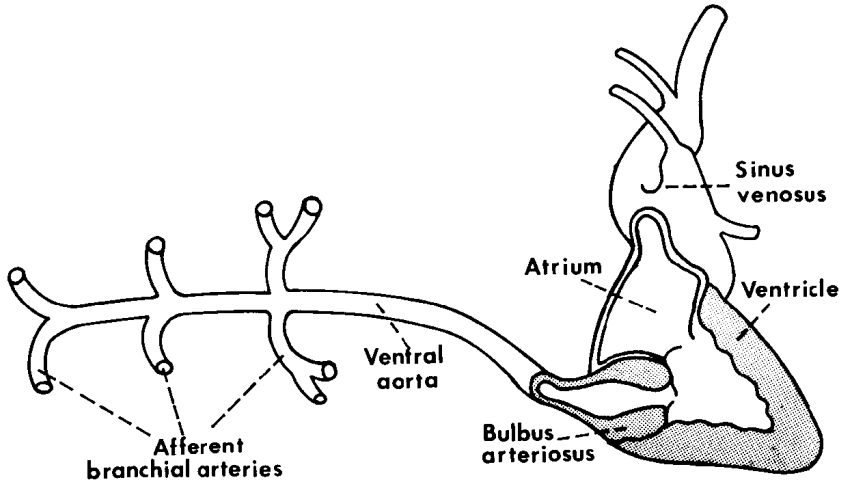

FIG. 1. A schematic representation of a salmonid heart and associated vasculature.

descriptions of the mechanical and electrical events of the cardiac cycle and other aspects of cardiac physiology have been presented in texts of fish physiology (140,158).

The bulbus arteriosus does not contain cardiac muscle or contract actively in sequence with the heart. It has been referred to by Satchell (158) as the "intrapericardial portion of the ventral aorta that balloons out to form [a] thick-walled onion-shaped chamber." Johansen (78) has called the bulbus "a swelling at the base of the heart, composed of arterial smooth muscle and elastic tissue." Regarding the distensibility of the tissue, Lansing (98) has remarked that "the organ is highly elastic, more so than any biological material previously encountered." Recent investigations on the innervation and vascularization of this organ have demonstrated the typical three layers found in vasculature, namely, the adventitia, media, and intima, and the presence of innervated smooth muscle cells within the media (192).

The bulbus arteriosus has been reported to act as an elastic reservoir to dampen the rise and fall of blood pressure as well as the fluctuations in flow rate (77). The crucial role of the tissue is not only to protect the gill capillaries from the pressure fluctuations, but to extend positive aortic flow through a larger portion of the cardiac cycle (78,140,158). Johansen (77) has reported that positive aortic flow exists in the ventral aorta of the cod for more than three-quarters of the cardiac cycle, whereas in mammals it is present for less than one-third. The importance of this flow prolongation is particularly significant when the relatively low heart rate of fish is considered: 30 beats/min in the cod (77), 21 in the carp (58), 32 in the Japanese eel, 46 in the rainbow trout (75), and 30 in largemouth bass (150).

Another function of the bulbus related to its incorporation into the pericardium and its intricate anatomical relationship with the atrium has been suggested (112,113). The bulbus is directly below the atrium, which is grooved on its ventral surface, and the two fit precisely together, emptying and filling reciprocally. From this arrangement, the bulbus modulates atrial activity and prevents the development of negative pressure within the pericardium (Holst, 1969 in ref. 158).

In a report discussing arterial system capacitance and capillary bed resistance in model mammalian circulatory system design, Taylor (184) has pointed out the good correlation of vascular system function with the mechanical properties of the tissue. Satchell (158) has extended circulatory system model designing to fish, which have two arterial systems in series, both distal from an intermittent pump action and placed on either side of a gill capillary bed. To achieve an even flow through this capillary resistance, Satchell has pointed out the requirements of having a diminished capacitance in the arterial system distal from the gills (dorsal aorta) and a large smoothing capacitance in the arterial system between the heart and the gills (ventral aorta). In other words, the model fish circulatory system should and, in fact, does have a large, compliant ventral aorta and a rigid, nondistensible dorsal aorta.

Additional studies on the histology and elastic modulus (the ratio of elastic tension developed to the amount of deformation that elicited it) support these functional characteristics in mammals and fish. Determinations of the elastic modulus in mammals have shown that vessels with a large ratio are rich in collagen, low in elastin, and have rather nondistensible walls, whereas vessels with low values are dominated by elastin and are easily distensible (153,184). These studies have also shown that elastin fibers are recruited by a very slight stretching of the wall and that the less extensible collagen is not stretched until the vessel is largely distended. Similar studies in sharks (Lander, 1964 and Learoyd, 1963 both cited in ref. 158) have shown that there is a gradient of diminishing elastin content from the proximal end of the ventral aorta through the afferent and efferent arteries up to the dorsal aorta. From the proximal to the distal end, the dorsal aorta is consistently low in elastin (9%) (mg/100 mg dried fat-free tissue $\times$ 100) and high in collagen (69%) (Lander, 1964 cited in ref. 158). The average elastin content in the ventral aorta was 31%, whereas collagen was 46%. Thus, the ventral aorta, which contains about three times more elastin than the dorsal aorta, is also relatively high in collagen. Values for rabbit thoracic aorta are 45 to 50% elastin and 20 to 25% collagen (184).

The ventral aorta of sharks consists of the typical three layers of tissue found in the vertebrate arterial wall (Lander, 1964 cited in ref. 158). The adventitia and media together constitute about one-eighth of the total wall thickness and are largely made up of collagen. The media is largely made up of circularly arranged elastin fibers with smooth muscle cells in between. By contrast, the adventitia of the dorsal aorta of the shark constitutes three-fourths of the total wall thickness and is extremely collagenous. There is no separate intimal layer and the remaining one-fourth is made up of the media layer, which has a twofold higher proportion of elastin than the whole wall. The dorsal aorta, which runs below and is attached to the spinal column, is not a symmetrical tubular vessel. The vessel wall is much thinner on the side facing the ventral surface of the vertebrae, and there is only a small amount of collagenous tissue with no circular muscle fiber in this region. The adventitia of the ventral surface of the dorsal aorta is "extended laterally to form a girdle of strands that lashes the aorta to the vertebrae" (158). Similar anatomical arrangements and histological features of the ventral and dorsal aortae have been described for the carp (44).

Investigations using perfused isolated trunk (201) and intact perfused gill (195) preparations from rainbow trout have demonstrated the large extent of passive distensibility in systemic and branchial circulations. Branchial vascular resistance is altered much more effectively by changing dorsal aortic pressure than by changing ventral aortic pressure. Autoregulatory mechanisms may also affect this resistance. The authors point out that there is considerable distensibility in the branchial vasculature, but that the systemic vascular resistance is more distensible at normal levels of blood pressure. This difference in distensibility may be less than these studies indicate, since a perfused head preparation with simulated normal dorsal aortic blood pressure was used to evaluate branchial vascular distensibility. Smith (170) described these branchial preparations as unaccountably sensitive to changes in outflow pressure and suggests that they are subject to disrupted flow rates.

THE PHYSIOLOGICAL PHARMACOLOGY OF FISH CARDIOVASCULAR SYSTEMS

As stated at the beginning of this chapter, knowledge of procedures used in pharmacology guides selection of approaches to toxicological investigations, especially those that are directed to understanding basic mechanisms of action. In order to focus attention on the procedures used to assess cardiovascular function in intact fish and to investigate control mechanisms of the major components of the system, this section is organized into four general subtopics. Described are studies on intact cardiovascular systems, isolated heart preparations, isolated gill preparations, and isolated vascular preparations. Within each subtopic, there is discussion of the relative effects of classes of neuronal and humoral compounds, in order to understand the pharmacological nature and magnitude of inherent responses in a given preparation.

Intact Cardiovascular Preparations

Experimental Design and Procedures

There has been considerable variation in experimental design and procedures since the first comprehensive study of pharmacological responses in the cardiovascular system of intact fish was conducted by MacKay (107). Skates weighing from 4 to 5 kg were immobilized by spinal cord transection or barbiturate anesthesia, and the ventral aorta was cannulated to record blood pressure. Drugs were injected into the lateral vein leading directly to the heart or into the gastric vein to observe the effects of hepatic circulation.

In the year subsequent to MacKay's report, the first major modification in surgical and cannulating procedures was presented (202). In addition to recording ventral aortic blood pressure from one of the branchial arteries, these workers cannulated the celiac artery and were able to monitor dorsal aortic blood pressure. This modification enabled them to observe changes in branchial as well as

systemic vascular resistance. Also, this modification did not alter the interpretation of the observations of the cardiac parameters measured by McKay (107), so a description of more cardiovascular responses of the intact system could be made.

In the years following these two pioneering reports, there have been many variations in experimental procedures. As in mammalian cardiovascular pharmacology, there has been continual application of new and improved technology. There have been major differences in surgical procedures, vasculature cannulated, approaches to restraint and anesthesia, expression of dosage, route of drug administration, and presentation of data.

Simultaneous recording of pre- and postbranchial blood pressures have increased the understanding of the control and regulation of branchial vascular resistance. Techniques for obtaining prebranchial blood pressure include the cannulation of the ventral aorta (15,86,114,177,195); of one of the afferent branchial ateries (20,79,146,189); of the bulbus arteriosus (32,33); and of one of the cardiac chambers (33). Postbranchial blood pressures have been obtained by direct cannulation of the dorsal aorta (15,33,195) or by cannulation of one of the major arteries branching from the dorsal aorta (32,79,113,146,177,189,195).

In other studies, blood pressure from only one vessel was recorded. Cannulation sites included the ventral aorta (194), the bulbus arteriosus (31,34), an afferent branchial artery (20), the caudal portion of the dorsal aorta (163,164,165,187), one of its branches (36,82,128), or an anterior section of the dorsal aorta (144,171).

Heart rate can easily be determined from blood pressure tracings and from electrocardiogram records (75,116,128,137,139). Cardiac output has been determined by indirect dilution methods (58,75,115), by direct measurements on the excised ventricle (60), and, more recently, by direct measurements on intact, functioning systems using a flow-probe around the major prebranchial vasculature (33,86,195). Electromagnetic probes have also been used to measure blood flow through the celiac artery, one of the major branches of the dorsal aorta (61).

In addition to *in vivo* studies, the effects of drugs on vascular resistance have been investigated by perfusion experiments on vascular segments *in situ*. Perfusion of the systemic vasculature has been conducted through the dorsal aorta (87,146,201,197,199) or one of its branches (31,34,59), while the branchial vasculature has been perfused through the ventral aorta (146,195,197) and the bulbus arteriosus (51). The perfused branchial and systemic preparations will be discussed in the isolated gill and isolated vasculature sections, respectively.

An artificially ventilated, perfused whole rainbow trout preparation has recently been described (200). These investigators perfused the anterior end of the sectioned ventral aorta and collected the venous effluent from the posterior end. Kent and Pierce (86) have used similar auto- and pump-perfused preparations of the dogfish shark.

There have also been differences reported in the intravascular route of administration of the drugs. These include intravenous injections that either lead directly to the heart (22,33,37,86, 107,113,202), or travel first through a portal circulation (26,31,32,107, 128,144,161,163,164,165,177,187,189,195). Intraarterial injections have also been used to administer drugs (79,82,86,146,171,194).

Pharmacology

Responses to Adrenergic and Cholinergic Agents

MacKay (107) observed that epinephrine (Epi) produces a marked, dose-related increase in ventral aortic blood pressure of the skate, which begins after a latent period of ½ to 1 min and reaches a maximum several min later. The duration of the response is long; a dose of 0.1 mg in a 4 kg skate lasts 1 to 2-½ hr. Epi increases the force of cardiac contraction while the rate decreases; injection through the hepatic circulation does not diminish the responses. Fish severely stressed by capture do not respond to Epi unless they rest for several days. The negative chronotropic response to Epi was later found to be part of a reflex mechanism mediated by the vagus nerve (114).